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Viral Gene Expression in Rat Trigeminal Ganglia Following Neonatal Infection With Varicella-Zoster Virus

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Newborn rats were injected intraperitoneally with uninfected human cells or cell infected with 56,000 pfu of varicella-zoster virus (VZV). Five to 6 weeks later, trigeminal ganglia were harvested and tested for VZV DNA and RNA by PCR. VZV gene 21 and 40 DNA were detected in most infected animals. Gene 21 RNA also was detected in ganglia from most infected animals, but not gene 40 RNA, paralleling previous observations in latently infected human ganglia. The neonatal rat may represent a useful new model for the study of VZV latency. ***J. Med. Virol. 58:286–290, 1999.*** Published 1999 Wiley-Liss, Inc.†

KEY WORDS: varicella-zoster; newborn rats; VZV DNA; VZV RNA; latency; ganglia

INTRODUCTION

Exposure of susceptible individuals to VZV results in a disseminated rash recognized clinically as varicella. Following resolution of the acute infection, the virus persists in a latent form in dorsal root ganglion cells. Reactivation results in herpes zoster manifested as a rash localized to the dermatome innervated by the neurons containing the reactivated virus.

Using molecular techniques to examine human trigeminal ganglia obtained at autopsy, attempts were made to detect gene products of 9 of the approximately 70 VZV genes; only 4 of these were expressed during latency [Cohrs et al., 1996]. The lack of practicable animal models, such as those that have been invaluable for studying herpes simplex virus (HSV) latency [Wagner and Bloom, 1997], has impeded the study of VZV latency. VZV genome has been detected in ganglia of guinea pigs inoculated with VZV, but the state of viral persistence and gene expression have not been characterized [Lowry et al., 1993]. In adult rats inoculated subcutaneously or in the footpad with VZV, DNA has been detected only in ganglia innervating the injection site. There was no evidence of dissemination [Sadzot-Delvaux et al., 1995].

In an effort to develop an alternative model for the study of VZV infection, newborn rats were tested as they have been reported to be far more susceptible than adult rats to infection with HSV [Tanaka and Southam, 1965] or coxsackie viruses [Xu and Cromwell, 1996]. In addition, human infants born to mothers who experience varicella during pregnancy [Brunell and Kotchmar, 1981] or infants who get varicella during the first month of life [Baba et al., 1986] often develop zoster in early life, suggesting that latency may be more readily established in newborns. Using RT-PCR, we screened the rat ganglia for RNA expressed by genes 21, a putative early gene, and gene 40, a late gene, as has been done in a previous study of human trigeminal ganglia. These were chosen as it had been postulated that there might be a block in transcription of late but not early genes during VZV latency [Cohrs et al., 1995].

MATERIALS AND METHODS

Infection

Ninth cell culture passage Emily strain of VZV, which was isolated in human melanoma cells from a child with a typical case of chicken pox, was propagated in human embryonic lung fibroblasts (HELF). Wistar rats were injected intraperitoneally with 0.03 cc of VZV-infected HELF or uninfected HELF within the first 48 hr of birth. Infected and control animals were housed in the same room in separate cages.

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Tissue Preparation and Extraction

Tissues removed from the rats were rinsed in PBS to remove blood and placed in a microtube surrounded by dry ice. The frozen tissues were triturated with a Kontes pellet pestle and nucleic acids were extracted using a DNA or an RNA isolation kit according to the manufacturer's directions (Puregene, Gentra Systems, Minneapolis, MN). The treated tissue was incubated overnight at 56°C in lysis solution containing 0.1 μg/ml of proteinase K (Sigma Chemical, St. Louis, MO) after which the tubes were vortexed and incubated an additional 3 hr. Proteinase K was inactivated by heating at 95°C for 15 min. The samples were chilled and then treated with RNase at 37°C for 15 min. Cellular proteins were precipitated, and then the DNA was precipitated from the supernatant by addition of isopropanol containing 0.3 μg/ml of glycogen (Boehringer Mannheim, Indianapolis, IN).

To extract RNA, proteinase K-treated lysates were mixed with DNA-protein precipitation solution (Puregene, Gentra Systems), and isopropanol containing 0.6-μg/ml glycogen was added to the supernatant to precipitate RNA.

VZV RNA and DNA were prepared from virus-infected HELF by the same method that was used to extract ganglia except that lysis solution was added directly to the monolayers instead of freezing the tissues.

Amplification of Nucleic Acids

Amplification of DNA was performed in a total volume of 50 μl containing 1 μl of rehydrated extracted DNA, 2.5 U of Taq DNA polymerase, 50-mM KCl, 1.5-mM $MgCl_2$, 20-mM Tris-HCl (pH 8.3; GIBCO-BRL, Bethesda, MD), 200 μM of each dNTP (Boehringer Mannheim), 5% glycerol, and 10 nM of 5′-TCACACACAATCGGATGTTGC-3′ and 5′-ATCGCTTGAGCATAGTGGTGG-3′, the sense and antisense primers, respectively, for gene 40 [Cohrs, et al., 1996], and 5′-AGCGTTGTAGCAGACGAGCAT-3′ and 5′ATGACAGCTTCCAACCCTGT-3′, the sense and antisense primers, respectively, for gene 21.

Reactions were performed using an automated cycler (Perkin Elmer Cetus, Emeryville, CA). After 35 cycles of denaturation at 94°C for 1 min followed by 1 min of annealing at 55°C and polymerization at 72°C for 1 min, there was a final polymerization cycle of 7 min. Ten μl of the product was separated by electrophoresis in a 1% agarose gel and transferred to Protean membranes (Type BA83, Schleicher & Schuell, Keene, NH).

The synthesis of cDNA was preceded by the digestion of DNA by incubation with Dnase I in reaction buffer (BRL, Gaithersburg, MD) containing 100-mM dithiothreitol and ribonuclease inhibitor (Boehringer Mannheim) for 10 min at 37°C followed by an additional incubation period of 20 min at 65°C to inactivate the DNase. The product was then incubated for 60 min at 39°C together with 2 U/μl of reverse transcriptase (MMLV, Gibco-BRL) in the manufacturer's reaction buffer, 100 pM of the downstream antisense primer, and 10 mM of each dNTP (Boehringer Mannheim). The product was then amplified with the appropriate primers as described above.

Southern Blots

A commercial kit (DuPont, Boston, MA) was used to random prime label probes with ^{32}P dCTP (DuPont). A 1.3-kb probe for gene 21 was obtained by digesting pBR322 containing the *Eco*RI N fragment of VZ DNA [Mishra et al., 1984] with *Eco*RI and *Bgl*II. For gene 40, a 447-bp fragment was obtained by amplifying the region of *Not*I B [Cohen and Seidel, 1993] using the probes described above. The products of these reactions were separated by gel electrophoresis and isolated using Geneclean II (Bio-100, La Jolla, CA). After prehybridization, hybridization, washing, and drying the Protean membranes containing the PCR products, they were placed in cassettes and exposed to film.

RESULTS

Infection of Neonatal Rats

All seven pups in one litter were injected intraperitoneally with uninfected HELF cells, and their trigeminal ganglia were harvested 14 to 42 days later. Each member of two other litters totaling 22 animals was inoculated intraperitoneally with HELF cells containing 56,000 pfu of VZV. Their ganglia were harvested 37 to 42 days later. One of the rats died the day following inoculation. None of the other infected or uninfected newborn rats showed obvious clinical evidence of VZV infection.

Persistence of VZV DNA in Neonatal Rat Ganglia

DNA was extracted from one trigeminal ganglion of every animal and amplified by PCR using primers specific for VZV genes 21 and 40. All seven control animals inoculated with uninfected HELF were negative by PCR for both VZV genes 21 and 40 DNA (Fig. 1; data not shown). Gene 21 DNA was detected in ganglia from 9 of the 10 rats in one infected litter and 9 of 12 rats of the other infected litter (Fig. 1A and B). Gene 40 DNA was also detected by PCR in 6 of 10 and 7 of 12 ganglia from these two infected litters (Fig. 1C and D). In no instance was gene 40 DNA detected in an animal that was not also positive for gene 21 DNA.

Expression of VZV Gene 21 but Not Gene 40 in Newborn Rat Trigeminal Ganglia

RT-PCR was performed on RNA extracted from the contralateral ganglia and neither gene 21 nor gene 40 RNA was detected in ganglia of rats inoculated with uninfected HELF (Fig. 2). In contrast, gene 21 RNA was found in 5 of the 10 animals in the first VZV-infected litter (Fig. 2A) and in 6 of 12 animals in the second infected litter (Fig. 2B). Gene 40 RNA was not found in any of the ganglia from the 22 VZV-infected rats (Fig. 2C and D).

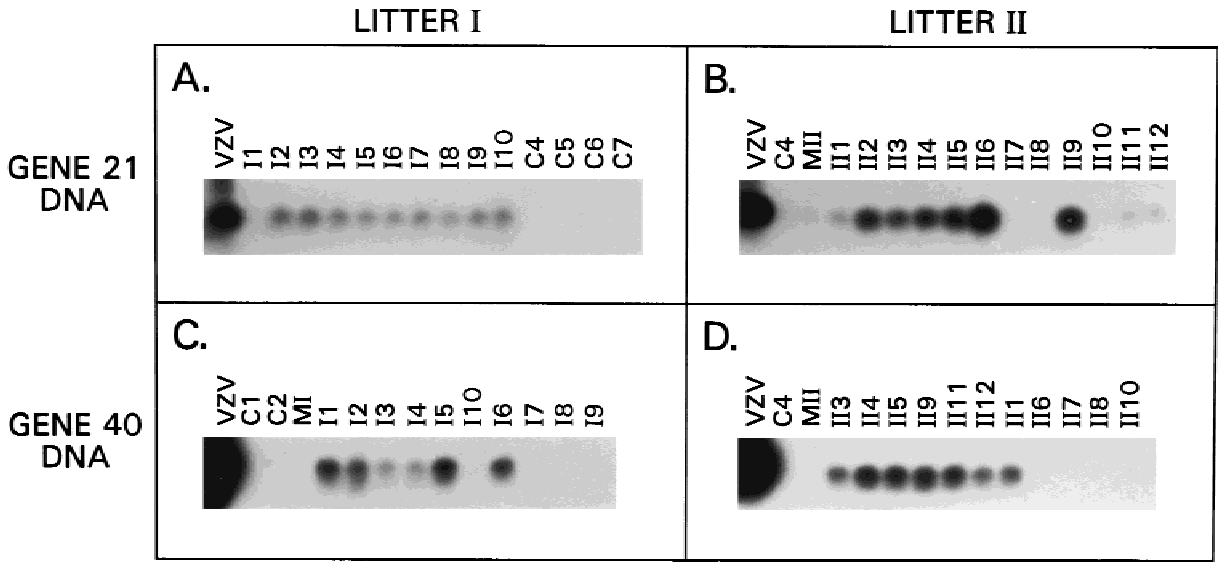

Fig. 1. Southern blots of VZV DNA detected by PCR analyses of trigeminal ganglia of VZV-infected neonatal rats sacrificed 37 to 42 days postinoculation and control rats inoculated 14 to 42 days postinoculation. Gene 21 sequences were detected in 9 of 10 rats in litter I (**A**), and 9 of 12 in litter II (**B**). Gene 40 sequences were detected in 6 of 10 rats in litter I (**C**) and 7 of 12 in litter II (**D**). Lanes labeled C1, C2, and C4 are from some of the uninfected control animal; M, from mothers of these rats. The lane labeled VZV contains VZV DNA extracted from infected tissue culture cells.

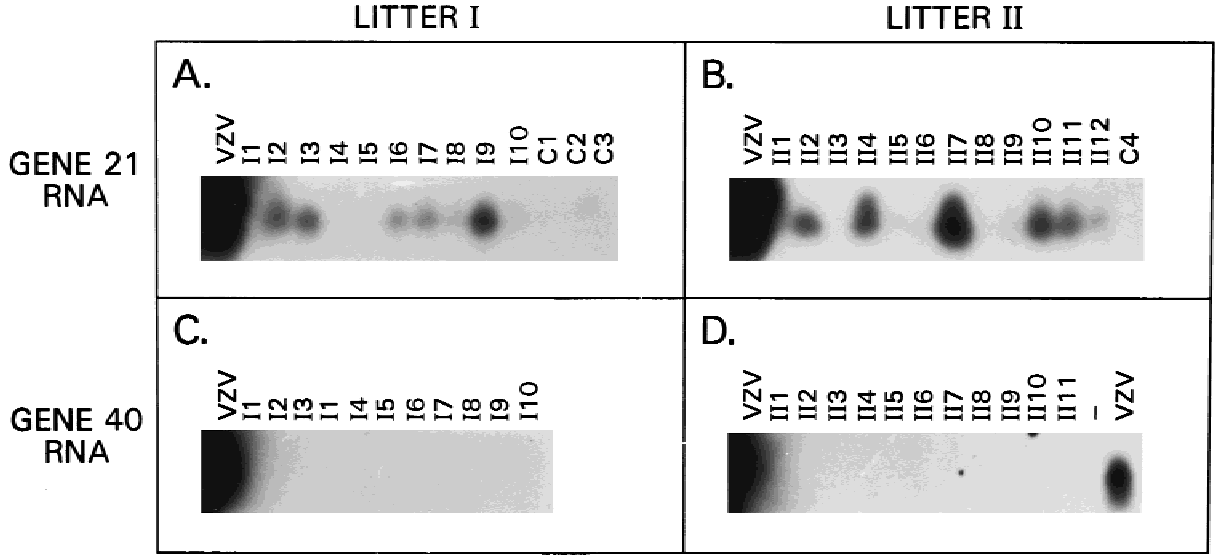

Fig. 2. Southern blots of RT-PCR products of RNA extracted from trigeminal ganglia of rats inoculated as newborns 37 to 42 days previously. Five of 10 rats in litter I (**A**) and 6 of 12 in litter II (**B**) were positive for VZV gene 21 cDNA. None of these 22 rats were positive for gene 40 cDNA (**C** and **D**). Lanes labeled C contains results of testing trigeminal ganglia from control pups; VZV contains cDNA from extracts of VZV-infected cells.

Inoculation of the Mothers

Each litter of rat pups was caged with its mother until they were weaned. After all of the infants were sacrificed, the three mothers were injected intraperitoneally with 168,000 pfu of VZV. Thirty-eight days later their ganglia were harvested and examined by PCR for VZV DNA and RNA; one was weakly positive for gene 21 DNA (Fig. 1B, MII). All of the remaining ganglia from the three adult rats were negative for both gene 21 and gene 40 RNA and DNA (data not shown).

DISCUSSION

These findings suggest that VZV can disseminate from the peritoneum to the trigeminal ganglia in neonatal rats. The VZV genome persisted in these ganglia for at least 5 weeks. Moreover, the pattern of gene expression emulates that seen in human latency, in that gene 21 RNA but not gene 40 RNA was detectable in trigeminal ganglia [Cohrs et al., 1995]. Gene 21 encodes a putative, early viral protein, whose homologue in HSV may participate in DNA replication. Gene 40

encodes a putative late, major capsid protein [Cohrs et al., 1995]. Thus, in the neonatal rat, as in humans, there appears to be a block in VZV replication in neural tissues after resolution of acute infection. Additional studies will be needed to test neonatal rat tissues for the presence of and the cellular localization of VZV gene 29, 62, and 63 RNA gene products, which have been detected in human ganglia [Croen et al., 1988; Mahalingam et al., 1990; Meier et al., 1993; Longu et al., 1998], and to determine which other genes may be expressed during VZV latency.

The relative ease with which VZV genome was detected in rat ganglia could prove invaluable in elucidating the pattern and regulation of latent gene expression. VZV DNA is much less abundant in human ganglia than is HSV DNA [Efstathiou et al., 1986]. Only 6 to 31 copies of VZV DNA were estimated by PCR to be present per 10^5 human ganglion cells [Mahalingam et al., 1993]. VZV RNA has been even more difficult to detect. In situ techniques are believed to underrepresent the number of RNA transcripts in trigeminal ganglion cells [Meier et al., 1993]. Thus, northern blot hybridization of highly enriched poly A^+ ganglion RNA [Meier et al., 1993] or hybridization of amplified cDNA libraries [Cohrs et al., 1996] has been used to study human ganglionic material. More recently, it has been possible to detect VZV proteins in trigeminal cells with antisera against immediate and early proteins [Longu et al., 1998]. Rat ganglia can be obtained more readily and more conveniently than can human autopsy material. RNA can be extracted promptly without the delay invariably associated with obtaining human material, reducing the possibility of postmortem reactivation [Meier et al., 1993]. Finally, the rat model can be exploited to study reactivation, immunologic factors involved in maintenance of latency, and the role of specific genes.

In the present experiments, VZV was exceedingly difficult to detect in trigeminal ganglia of the adult rats inoculated intraperitoneally with a dose of virus 3 times that given to the newborns and approximately 120 times the dose used to immunize humans against varicella. Only one of the ganglia obtained from the adult rats was weakly positive for gene 21 DNA (Fig. 1B, MII). The remaining tests for genes 40 DNA (Fig. 1C, MI, Fig. 1D, MII), gene 21 DNA, and all RNA assays were negative (data not shown). Others have reported that VZV genome could be detected in adult rats only in the ganglia innervating the inoculated dermatome. Thus, they hypothesized that, as with HSV [Knotts et al., 1974; Wagner and Bloom, 1997], VZV reached the ganglia by traveling up the neuronal axon [Sadzot-Delvaux et al., 1995]. The larger proportion of the pups infected and the greater abundance of VZV DNA in their trigeminal ganglia suggest that newborn rats may be more susceptible to infection with VZV than adult rats.

VZV DNA was demonstrable in the trigeminal ganglia following intraperitoneal injection of rats during the newborn period. As the trigeminal ganglia do not innervate the peritoneum, it is likely that infection of the newborn's trigeminal ganglia was the result of a disseminated infection. In preliminary experiments, VZV DNA was found in dorsal and trigeminal ganglia as early as 6 days postinoculation (data not shown). In these preliminary experiments, the VZV genome was detected in ganglia of newborn rats that were inoculated with as little at 200 pfu of cell-free virus. In the current experiments, pups were inoculated with 56,000 pfu of VZV cell-associated virus, approximately 40 times the dose of cell-free VZV used to immunize humans. It has not been determined whether the early-passage wild strain used in these experiments is more likely to infect ganglia than laboratory or attenuated vaccine strains.

In summary, we have shown that newborn rats are readily infected with VZV and that the virus disseminates and produces a persistent infection of the trigeminal ganglia. Thus, the newborn rat may prove to be a useful model for studying VZV latency, pathogenesis, and host factors involved in VZV infection.

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